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A DISCRIMINANT FUNCTION ANALYSIS OF THE FROGS OF THE GENUS *ADENOMERA* (AMPHIBIA: LEPTODACTYLIDAE)

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A previous analysis of the systematics of the frogs of the genus *Adenomera* (Heyer, 1973) was completed without benefit of field experience or computer analysis. I have now had field experience with three species in the complex and have also learned that in at least one case, one of the species previously described is a composite of two species. The purpose of this paper is to apply a multivariate technique of analysis to data previously used in a variable by variable analysis to learn which method best determines: (1) species limits; and (2) patterns of geographic variation.

Methods and Materials

The original data from the previous study (Heyer, 1973) are treated as follows. Data are used only for adults where complete information is available. The six variables of the original study are treated for computer analysis as follows. A) Snout-vent length (size) is entered as originally recorded. B) Dorsal pattern is not used because the states recognized previously do not show an orderly progression (Heyer, 1973, Fig. 2). The character has four more or less distinct patterns, dark striped, uniform, symmetrically spotted, asymmetrically spotted. In order for correlations to be made, which the discriminant function analysis does, the states must have a meaningful relationship to each other. That is, if the four pattern states were

coded as 1, 2, 3, 4, this implies that state 2 is derivable from state 3, or vice versa, etc. As simple inspection of the states does not allow relationships, derivability, or progressions to be made, and as there is no genetic information on the inheritance of color pattern for these frogs, the character is omitted from computer analysis. C) The three states of the dorsolateral stripes are coded as 1, no stripes; 2, light, narrow, stripes from eye to inguinal region; 3, light, broad, conspicuous stripes from eye to inguinal region. D) The original seven states recognized for middorsal stripes reduce to three states for computer analysis: 1, no stripe; 2, pin stripe extending from vent to sacrum or beyond; 3, broad stripe from vent to tip of snout. E) Snout shape ratio is entered as originally recorded (see Heyer, 1973, for determination of this ratio). F) The four states of toe tip expansion are coded from no expansion, 1, to large distinct disks, 4, according to the previous coding scheme (Heyer, 1973, Fig. 1). In the original study, intermediate toe tip categories were recorded. For coding purposes, wherever an intermediate state is encountered, the first state recorded is used (e.g. the intermediate state A–B as previously recorded would be coded as 1 here where A = 1, B = 2).

The data are analyzed using the BMD07M program, Stepwise Discriminant Analysis (Dixon, 1974). The use of discrete variables places the following restriction on interpretation of the results. The discriminant function analysis uses correlations. While calculation of correlation coefficients does not require normality, normality insures a valid test of significance if applied. (Having non-normally distributed data does not mean that a significance test is necessarily invalid, however.) As the variables used here are not all normally distributed, a statistical interpretation of the results is open to question. The immediate consequence of this is that the statistical information provided in the entering order of the variables should not be used as given. The first variable entered is that which has the greatest intergroup variation and the least intragroup variation. The second variable entered is that which has the next greatest intergroup variation and correlates best with the first variable entered, etc. Thus, the entering order of the variables provides important information. The program determines an approxi-

mate F statistic for each step so that a statistical determination can be made on which variables are adding information to the analysis and which are not; because discrete variables are used in this study, this information can not be statistically interpreted with certainty. This restriction does not invalidate the analysis itself. Correlation coefficients for non-normally distributed data are valid as long as there is a relationship among the data elements. For systematic studies, this restriction is not serious. The results of the analysis described here are valid; the results are repeatable. If someone else collected the data in the same way, the results would be the same. An unknown can be entered and classified. The results can be interpreted in biological terms, the only restriction is that statistical confidence limits or other statistical interpretations can not be made on or from the results.

The discriminant function program produces several data analyses and formats of results. Three portions of the discriminant function analysis are used in this study: (1) order of variables entered, (2) canonical variable analysis, (3) posterior classification of cases into groups. The order of variable entering has been commented on above. The first two canonical variables are plotted against each other, and it is the graphed results which are used here. For each data card entry, a posterior probability of belonging to each of the groups is determined and a classification based on this analysis is produced. For further explanation of terms, logic, and statistical procedures, see Dixon (1974).

Because there is sexual dimorphism in at least one character (size), data for males and females are analyzed separately.

Results of Analysis

Species Limits: The discriminant function analysis requires that the groups be known on which the analysis takes place. In this case, the data cards are arranged into groups corresponding with the species limits previously recognized, that is, the species *Adenomera andreae*, *bokermanni*, *hylaedactyla*, *marmorata*, and *martinezi*. Not enough data are available for the recently described *A. lutzi* (Heyer, 1975) for computer analysis and only enough data for females of *martinezi* are available for analysis.

For females, sample sizes for the groups are: *andreae*, 197; *bokermanni*, 27; *hylaedactyla*, 175; *marmorata*, 102; *martinezi*, 14. The variables

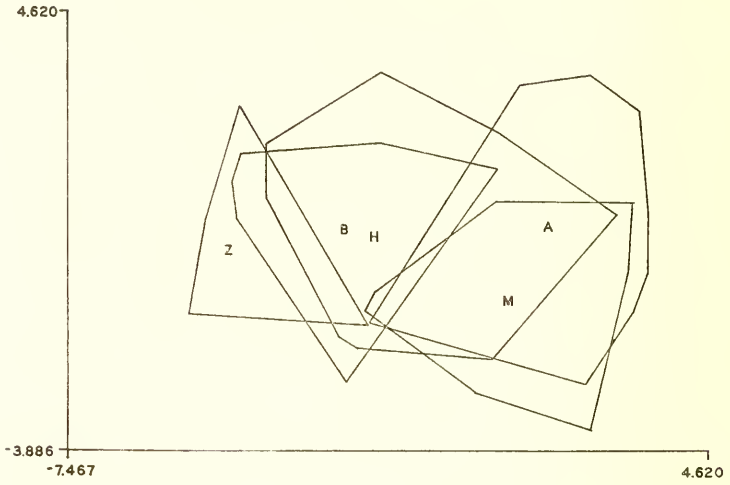

Fig. 1. Plot of first against second canonical variables for females of the genus *Adenomera*. A = *A. andreae*, B = *A. bokermanni*, H = *A. hylaedactyla*, M = *A. marmorata*, Z = *A. martinezi*. Letters are placed at group means. Envelopes contain all group members.

enter in the following order: toe disks, size, snout shape, middorsal stripe, dorsolateral stripe. Almost all of the variation is accounted for by the first variable (F values can give a comparative idea, although can not be statistically interpreted. The F value for the first variable entered, toe disks, is 346, the F value for the next step entered, size, is 17; remaining F values are smaller). The results of the canonical analysis (Fig. 1) show moderate, but certainly not complete, separation of the groups. The first canonical variable accounts for 90% of the total dispersion, the first two canonical variables account for 97% of the total dispersion. The posterior classification of cases into group results (Table 1) clarifies the canonical analysis as presented in Fig. 1. It is clear that the five variables are sufficient to separate *martinezi* from all the others, *andreae* from *hylaedactyla*, and *bokermanni* from *marmorata*. Most classification errors involve *bokermanni–hylaedactyla* and *andreae–marmorata*.

For males, sample sizes for the groups are: *andreae*, 73; *bokermanni*, 29; *hylaedactyla*, 76; and *marmorata*, 80. The variables enter in the following order: toe disks, size, middorsal stripe, dorsolateral stripe, snout shape. Almost all of the variation is accounted for by the first variable with virtually all variation accounted for by the first two variables. The canonical analysis results (Fig. 2) are similar to the female results. The first canonical variable accounts for 85% of the

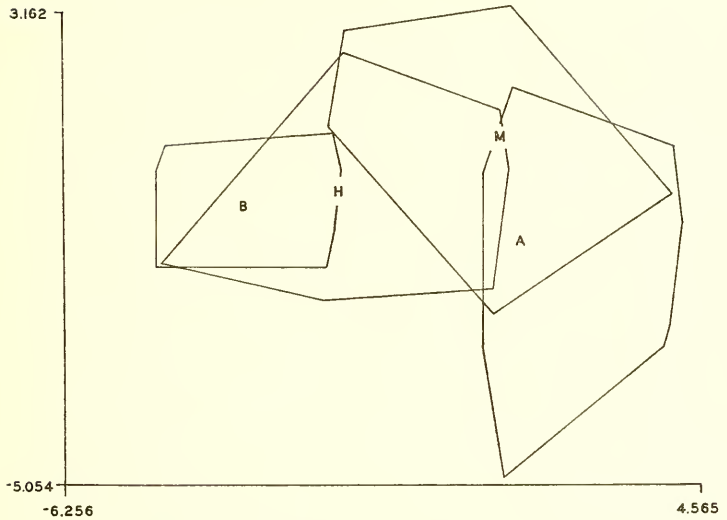

FIG. 2. Plot of first against second canonical variables for males of the genus *Adenomera*. A = *A. andreae*, B = *A. bokermanni*, H = *A. hylaedactyla*, M = *A. marmorata*. Letters are placed at group means. Envelopes contain all group members.

total dispersion; the first two canonical variables account for 99% of the total dispersion. The posterior classification (Table 2), while generally comparable to the female results is different in two ways. First, more males are correctly classified to group than females. This indicates that males are easier to identify than females for the variables used. Second, while the separation of *andreae* from *marmorata* involves the same kind of classification errors as for females, the same is not true for *boker-*

TABLE 1. Posterior classification of females of the genus *Adenomera*.

Group	Number (percent) of cases classified into group				
	martinezi	*andreae*	*hylaedactyla*	*marmorata*	*bokermanni*
martinezi	13(93)	0	0	0	1 (7)
andreae	0	145(74)	1 (0)	51(26)	0
hylaedactyla	5 (3)	3 (2)	114(65)	9 (5)	44(25)
marmorata	0	19(19)	2 (2)	81(79)	0
bokermanni	4(15)	1 (4)	8(30)	0	14(52)

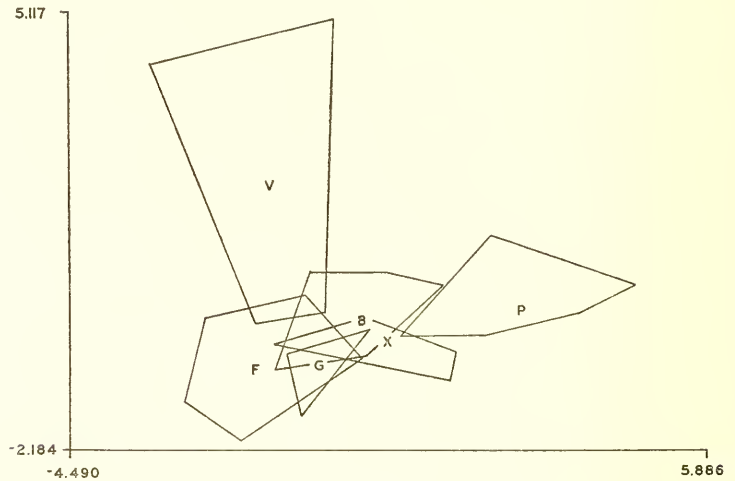

FIG. 3. Plot of first against second canonical variables for geographic samples of males of *Adenomera hylaedactyla*. B = Bolivia, Santa Cruz; X = Brasil, Amazonas; V = Brasil, Rondônia; F = French Guiana; G = Guyana, Essequibo; P = Peru, Huanuco. Letters are placed at group means. Envelopes contain all group members.

manni and *hylaedactyla*. Very few *hylaedactyla* are classified as *bokermanni*, while many *bokermanni* are classified as *hylaedactyla*.

In an analysis of this sort, the groups should be completely separated by the graphic canonical variable analysis (e.g. Figs. 1 and 2) and the posterior classification should yield 100% correct classifications. The results of this analysis are not this clear-cut or convincing. The following recently gathered information is pertinent to a meaningful interpretation of the results. I have had the opportunity to have field experience with the following three taxa as used previously (Heyer, 1973): *A. an-*

TABLE 2. Posterior classification of males of the genus *Adenomera*.

	Number (percent) of cases classified into group			
Group	*andreae*	*hylaedactyla*	*marmorata*	*bokermanni*
andreae	59(81)	0	13(18)	1 (1)
hylaedactyla	3 (4)	68(89)	3 (4)	2 (3)
marmorata	12(15)	3 (4)	65(81)	0
bokermanni	0	13(45)	0	16(55)

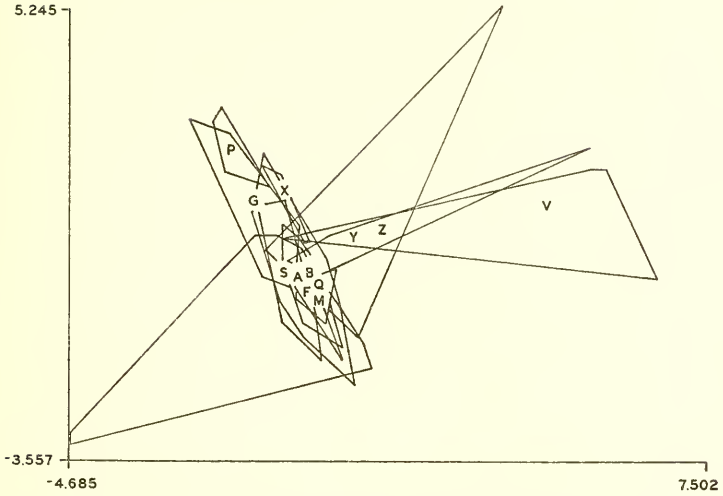

Fig. 4. Plot of first against second canonical variables for geographic samples of females of *Adenomera hylaedactyla*. A = Bolivia, Beni; B = Bolivia, Santa Cruz; Z = Brasil, Acre; Y = Brasil, Amapa; X = Brasil, Amazonas; V = Brasil, Rondônia; F = French Guiana; G = Guyana, Essequibo; Q = Peru, Junin; P = Peru, Pasco; S = Surinam; M = Venezuela, Monagas. Letters are placed at group means. Envelopes contain all group members.

dreae, *hylaedactyla*, and *marmorata*. *Adenomera andreae* and *hylaedactyla* occur together over a wide geographic area: *A. andreae* is a diurnal forest floor species, *A. hylaedactyla* is a nocturnal open formation species. There is no confusing these species in the field. The results of this study show that the two species are morphologically separable also. *Adenomera marmorata* was observed in the state of São Paulo. The species occurs both in forest and open formations (heavy grass) and calling occurs during and after rains irrespective of time. I am convinced that *andreae* and *marmorata* are distinct species, although the results of this study indicate that there is a fair amount of morphological overlap between them. Werner C. A. Bokermann and Eugenio Izecksohn (pers. comms.) have informed me that the species I described as *bokermanni* is a composite of two morphologically similar species. Data for the males (Table 2) show this quite clearly. Because two species are involved, the posterior classification into groups was poor for *bokermanni*. The posterior identification errors between *andreae* and *marmorata* suggest that *marmorata* may also be a composite species, but the evidence is not as clear as for *bokermanni*. Nothing

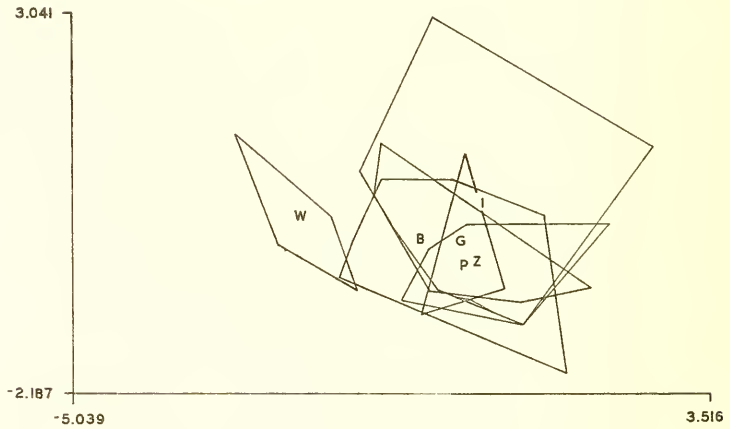

Fig. 5. Plot of first against second canonical variables for geographic samples of males of *Adenomera andreae*. B = Bolivia, Santa Cruz; W = Brasil, Para; 2 = Ecuador, Napo; 1 = Ecuador, Pastaza; G = Guyana, Essequibo; P = Peru, Loreto. Letters and numbers are placed at group means. Envelopes contain all group members.

further can be done presently with the data to resolve these questions as the specimens are no longer at hand and sample sizes for *bokermanni* and *marmorata* are too small to analyze on a geographic basis as is done for *andreae* and *hylaedactyla*.

Geographic Variation: For analysis of geographic variation, each group consists of at least three specimens from a single locality. For only two species are there enough specimens from enough localities to analyze in this way.

The following localities (specific locality information not included here as it is not necessary for present purposes) and sample sizes comprise the groups for male *A. hylaedactyla*: Bolivia, Santa Cruz, 9; Brasil, Amazonas, 4; Brasil, Rondônia, 6; French Guiana, 11; Guyana, Essequibo, 4; Peru, Huanuco, 7. The order of variable entering (and F values, again included to add a dimension of importance, not statistical significance) is: size (12.8), toe disks (5.5), snout shape (4.0), mid-dorsal stripe (1.0), dorsolateral stripes (0.6). Any patterns of variation should be demonstrated by the canonical variable analysis (Fig. 3). The first two canonical variables describe 94% of the total dispersion, the first variable accounting for 65%. The samples from Rondônia and Peru are distinctive from the other samples and distinctive from each other (Fig. 3).

The following localities and sample sizes comprise the groups for female *A. hylaedactyla*: Bolivia, Beni, 24; Bolivia, Santa Cruz, 12; Brasil,

Acre, 5; Brasil, Amapa, 4; Brasil, Amazonas, 3; Brasil, Rondônia, 5; French Guiana, 51; Guyana, Essequibo, 7; Peru, Junin, 4; Peru, Pasco, 5; Surinam, 5; Venezuela, Monagas, 6. The order of variable entering and (F values) is: toe disks (11.3), size (7.2), middorsal stripe (4.2), snout shape (2.2), and dorsolateral stripe (1.6). The first canonical variable accounts for 45% of the total dispersion, the first two variables account for 78% (Fig. 4). The sample from Brasil, Rondônia is most distinctive, the populations from Brasil, Amapa and Brasil, Acre are moderately distinct from other samples and similar between themselves.

The differences in variable entering and graphic representation of canonical variables may be due to the different sample sizes involved. No meaningful pattern of geographic variation is evident for either male or female *A. hylaedactyla* from the results as represented in Figs. 3 and 4.

The following localities and sample sizes comprise the groups for male *A. andreae*: Bolivia, Santa Cruz, 12; Brasil, Para, 5; Ecuador, Napo, 7; Ecuador, Pastaza, 18; Guyana, Essequibo, 6; Peru, Loreto, 5. The order of variable entering (and F values) is: size (4.6), snout shape (1.0), middorsal stripe (0.8), toe disks (0.6), dorsolateral stripes (0.6). The first canonical variable accounts for 72% of the total dispersion, the first two canonical variables account for 87%. The sample from Brasil, Para is distinctive (Fig. 5).

The following localities and sample sizes comprise the groups for female *A. andreae*: Bolivia, Santa Cruz, 16; Brasil, Amapa, 5; Brasil, Amazonas, 6; Brasil, Amazonas, 15; Brasil, Para, 18; Brasil, Rondônia, 4; Colombia, Meta, 6; Ecuador, Moruna, 8; Ecuador, Napo, 10; Ecuador, Napo, 23; Ecuador, Pastaza, 5; Ecuador, Pastaza, 10; Ecuador, Pastaza, 6; French Guiana, 5; Guyana, Essequibo, 8; Peru, Loreto, 6; Surinam, Marowijne, 4; Surinam, Suriname, 8. The order of variable entering (and F values) is: size (10.9), middorsal stripe (3.8), snout shape (2.6), toe disks (2.4), dorsolateral stripes (1.8). The first canonical variable accounts for 54% of the total dispersion, the first two account for 74%. No clear pattern of geographic variation is evident from the plot of the first two canonical variables (Fig. 6).

The results for male and female *A. andreae* are similar. No pattern of geographic variation is evident from the results as represented in Figs. 5 and 6.

Discussion

The number of variables used in this analysis is minimal. Except for dorsal pattern, which poses a coding problem, they are the only variables available for analysis from external morphology because all of the frogs of this study have a basic morphological similarity. For the kinds of characters available in frogs, use of multivariate techniques is concluded to be suitable in determining species limits but not in evaluating patterns of geographic variation. As the earlier study (Heyer,

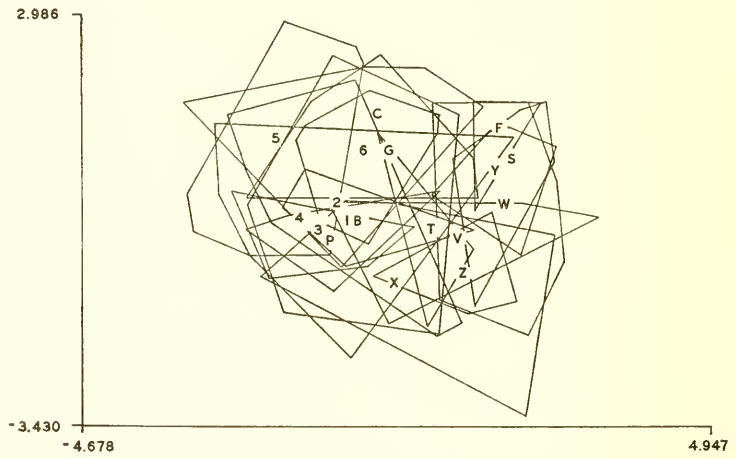

Fig. 6. Plot of first against second canonical variables for geographic samples of females of *Adenomera andreae*. B = Bolivia, Santa Cruz; Z = Brasil, Amapa; Y = Brasil, Amazonas; X = Brasil, Amazonas; W = Brasil, Para; V = Brasil, Rondônia; C = Colombia, Meta; 6 = Ecuador, Moruna; 5 = Ecuador, Napo; 4 = Ecuador, Napo; 3 = Ecuador, Pastaza; 2 = Ecuador, Pastaza; 1 = Ecuador, Pastaza; F = French Guiana; G = Guyana, Essequibo; P = Peru, Loreto; T = Surinam, Marowijne; S = Surinam, Suriname. Letters and numbers are placed at group means. Envelopes contain all group members.

1973) found patterns of geographic variation, a more detailed comparison is necessary to determine why this study differs in this respect from the previous one.

Previously, the characters of dorsal pattern, dorsolateral stripes, and middorsal stripes where shown to demonstrate meaningful geographic variation in *A. hylaedactyla* (Heyer, 1973). In this study, dorsal pattern information could not be coded and the information content of middorsal stripes was greatly reduced in coding for computer analysis. The characters which had the greatest intergroup variation in this study are size and toe disks; it is not surprising that these characters do not show any patterns of geographic variation here as they were previously found not to vary geographically. For *A. andreae*, the previous analysis (Heyer, 1973) showed that dorsal pattern, middorsal stripe pattern and size varied geographically. Only one of these, size, shows much intergroup variation in the present analysis, but the results are not interpretable in terms of geographic variation.

Most of the information which demonstrated geographic variation in the previous study could not be employed in this analysis due to re-

strictions in the kinds of character states that can be used in a discriminant function analysis or any other in which the basic analytic method involves correlations. For the kinds of data available on certain groups of frogs, it would appear that the best use of multivariate techniques is to aid in species limit determinations, but not in describing geographic variation within a species.

Acknowledgments

Dr. Charles D. Roberts, Smithsonian Institution, has been extremely patient and helpful in introducing me to the use of multivariate analyses. I thank Dr. P. E. Vanzolini for pointing out several limitations involved in using multivariate techniques; we still differ with respect to whether discrete variables can be validly analyzed by multivariate techniques, however. Ms. Susan Arnold transformed the data from the original form to computer cards. Dr. George R. Zug criticized the manuscript.

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